

Week 0 Notebook

(Pre-Program Work)

May 6, 2015

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Transformation

Performed a transformation of competent *E. coli* per iGEM protocols except:

- 10 ul competent cells used
- 1 ul plasmid used
- 15 minutes on ice
- 2014 plates used along with 2015 efficiency test kit
- used LB instead of SOC
- 30 min recovery
- streak to Cam plates (full volume)
- incubate 37°C overnight

Plasmids transformed:

- K847060 Plate 2 well 5G
- K847061 Plate 1 well 24C
- K847004 Plate 1 well 24E
- K652004 Plate 1 well 10C
- + transformation efficiency test (0.5, 5, 10, 20, +50 pg) (RFP)

May 7, 2015

Charlotte Reames, Natalie Farny

Plate check, 11:15 am

Number of colonies:

- RFP 0.5 pg/ml: 1
- RFP 5 pg/ml: 1
- RFP 10 pg/ml: 27
- RFP 20 pg/ml: 3
- RFP 50 pg/ml: 23
- K847060 (OtsA): 12
- K47061 (OtsB): 2
- K847004 (SodCuZn): 13
- K652004 (ZeAFP): 16

RFP test plates returned to incubator.

One colony from each of the other plates was picked to grow for a miniprep, placed in 5 ml LB liquid media + Cam into 37°C shaker.

May 8, 2015

Charlotte Reames, Natalie Farny

Plate check, 2:00 pm

RPF plates, number of colonies:

- 0.5 pg/ml: 28
- 5 pg/ml: 12
- 10 pg/ml: 135
- 20 pg/ml: 92
- 50 pg/ml: 174

Miniprep: per Macherey-Nagel protocol 5.1
for 15-1C, 15-2C, 15-3C, 15-4C.

May 27, 2015

David Morgan

Plate pouring and transformation

- A set of Amp LB plates were poured
- *E. coli* were transformed using the plasmids on the back of the page. Where 14/15 was an option the 2015 kit was used.
- 30 ul of competent cells were used
 - This utilized the remainder of our competent cell stocks; more are required.
- 1 ul of plasmid used
- 30 min on ice
- Heat shock: 37°C for 90s
- 30 min recovery in 37°C shaker
- Full volume plated
- Incubated 37°C overnight

May 28, 2015

David Morgan

Inoculating liquid cultures

- 5 ml liquid LB
- 5 ul antibiotic (Cam or Amp)
- J23109 & J23111 were left to grow more overnight, as no visible colonies were present.
- Tube lids were left loose and taped down to allow a more aerobic environment.
- Tube were plated in 37°C shaker overnight.
- Plates were placed back in the incubator at 37°C.

Device	# of colonies
BBa_E0432	>150
BBa_E0422	29
J23108	70
I13504	>150
J23109	0
J23111	0

May 29, 2015

David Morgan

Miniprep

- Conducted according to the miniprep protocol
- All plates grew further, the results are recorded here.
- Minipreps stored at 4°C

Device	# of Colonies
J23109	>75
J23111	23
All Others	>150